

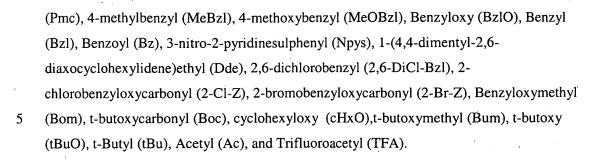
WHAT IS CLAIMED IS:

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- A fluorogenic composition comprising a polypeptide backbone or a nucleic acid backbone joining two fluorophores of the same species whereby said
 fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores.
 - 2. The fluorogenic composition of claim 1, wherein said composition comprises a polypeptide backbone.
- 3. The fluorogenic composition of claim 2, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.
 - 4. The fluorogenic composition of claim 2, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.
 - 5. The fluorogenic composition of claim 2, wherein said polypeptide backbone ranges in length from about 4 to about 31 amino acids.
 - 6. The fluorogenic composition of claim 2, wherein said composition is attached to a solid support.
- 7. The fluorogenic composition of claim 2, wherein said composition is inside a mammalian cell.
 - 8. The fluorogenic composition of claim 2, wherein said composition bears a hydrophobic group.

The fluorogenic composition of claim 8, wherein said hydrophobic

group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1
fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl



- 10. The composition of claim 9, wherein said hydrophobic group is Fmoc.
 - 11. The composition of claim 9, wherein said hydrophobic group is Fa.
- 12. The composition of claim 9, wherein said hydrophobic group is attached to the amino terminus of the molecule.
- 13. The fluorogenic composition of claim 1, wherein said composition comprises a nucleic acid backbone.
- 14. The fluorogenic composition of claim 13, wherein said nucleic acid backbone comprises a restriction site.
- 15. The fluorogenic composition of claim 13, wherein said nucleic acid backbone is self-complementary and forms a hairpin.
- 16. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.
- 20 17. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.
 - 18. The fluorogenic composition of claim 13, wherein said composition is attached to a solid support.
- 19. The fluorogenic composition of claim 13, wherein said composition is inside a mammalian cell.
 - 20. The fluorogenic composition of claim 13, wherein said composition bears a hydrophobic group.

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- 21. The fluorogenic composition of claim 20, wherein said hydrophobic group is selected from the group consisting of Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).
- 22. The composition of claim 21, wherein said hydrophobic group is Fmoc.
 - 23. The composition of claim 21, wherein said hydrophobic group is Fa.
- 24. The fluorogenic composition of claim 1, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.
- 25. The fluorogenic composition of claim 1, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
- 26. The fluorogenic composition of claim 1, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
 - 27. The fluorogenic composition of claim 1, wherein said fluorophores are carboxytetramethylrhodamine.
- 25 28. The fluorogenic composition of claim 1, wherein said fluorophores are carboxyrhodamine-X.
 - 29. The fluorogenic composition of claim 1, wherein said fluorophores are carboxyrhodamine 110.

- 30. The fluorogenic composition of claim 1, wherein said fluorophores are diethylaminocoumarin.
- 31. The fluorogenic composition of claim 1, wherein said fluorophores are carbocyanine dyes.
- 5 32. A mammalian cell comprising a fluorogenic composition comprising a polypeptide backbone or a nucleic acid backbone joining two identical fluorophores whereby said fluorophores form an H-dimer resulting in the quenching of the fluorescence of said fluorophores.
- 33. The cell of claim 32, wherein said composition comprises a 10 polypeptide backbone.
 - 34. The cell of claim 32, wherein said composition comprises a nucleic acid backbone.
 - 35. The cell of claim 32, wherein said composition bears a hydrophobic group.
- 36. The cell of claim 35, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl 20 (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), 25 cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).
 - 37. The cell of claim 36, wherein said hydrophobic group is Fmoc.
 - 38. The cell of claim 32, wherein said hydrophobic group is Fa.

- 39. The cell of claim 32, wherein said hydrophobic group is attached to the amino terminus of the molecule.
- 40. The cell of claim 32, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.
- 5 41. The cell of claim 32, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
 - 42. The cell of claim 32, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
- 10 43. The cell of claim 42, wherein said fluorophores are carboxytetramethylrhodamine.
 - 44. The cell of claim 42, wherein said fluorophores are carboxyrhodamine-X.
- 45. The cell of claim 42, wherein said fluorophores are carboxyrhodamine 110.
 - 46. The cell of claim 42, wherein said fluorophores are diethylaminocoumarin.
 - 47. The cell of claim 42, wherein said fluorophores are carbocyanine dyes.
- 20 48. A method of detecting the activity of a protease, said method comprising:
 - i) contacting said protease with a fluorogenic composition comprising a polypeptide backbone joining two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and
 - ii) detecting a change in fluorescence or absorbance of said fluorogenic composition where an increase in fluorescence or a change in absorbance indicates that said protease cleaves said polypeptide backbone.

- 49. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.
- 50. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.
- 5 51. The method of claim 48, wherein said composition is attached to a solid support.
 - 52. The method of claim 48, wherein said composition is inside a mammalian cell.
- 53. The method of claim 48, wherein said composition is inside a insect cell.
 - 54. The method of claim 48, wherein said composition is inside a yeast cell.
 - 55. The method of claim 48, wherein said composition bears a hydrophobic group.
- 15 56. The method of claim 48, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde),
- bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), 25 cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-

57. The method of claim 56, wherein said hydrophobic group is attached to the amino terminus of the molecule.

- 58. The method of claim 48, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.
- 59. The method of claim 48, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
- 5 60. The method of claim 48, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
 - 61. The method of claim 48, wherein said contacting is in a histological section.
 - 62. The method of claim 48, wherein said contacting is in a cell culture.
 - 63. The method of claim 48, wherein said contacting is contacting a seeded or cultured adherent cell.
 - 64. The method of claim 48, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.
 - 65. The method of claim 48, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, confocal microscopy, fluorescence microplate reader, flow cytometry, fluorometry, and absorption spectroscopy.
- 66. A method of detecting the activity of a nuclease or the presence of a nucleic acid, said method comprising:
 - i) contacting said nuclease or said nucleic acid with a fluorogenic composition comprising a nucleic acid backbone joining two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and
- 25 ii) detecting a change in fluorescence or absorbance of said fluorogenic composition where an increase in fluorescence or a change in absorbance indicates that said nuclease cleaves said nucleic acid backbone or that said nucleic acid hybridizes to said backbone.

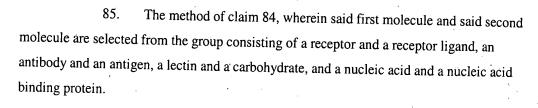


- 67. The method of claim 66, wherein said nucleic acid backbone comprises a restriction site.
- 68. The method of claim 66, wherein said nucleic acid backbone is self-complementary and forms a hairpin.
- 5 69. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.
 - 70. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.
- 71. The method of claim 66, wherein said composition is attached to a solid support.
 - 72. The method of claim 66, wherein said composition is inside a mammalian cell.
 - 73. The method of claim 66, wherein said composition is in solution.
 - 74. The method of claim 66, wherein said composition bears a hydrophobic group.
 - 75. The method of claim 74, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-
- bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

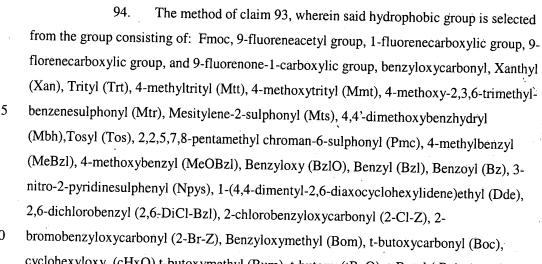
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- 76. The method of claim 66, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.
- 77. The method of claim 66, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
- The method of claim 66, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
 - 79. The method of claim 66, wherein said contacting is in a histological section.
 - 80. The method of claim 66, wherein said contacting is in a cell culture.
 - 81. The method of claim 66, wherein said contacting is contacting a seeded or cultured adherent cell.
 - 82. The method of claim 66, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.
 - 83. The method of claim 66, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, confocal microscopy, fluorescence microplate reader, flow cytometry, fluorometry, and absorption spectroscopy.
- 84. A method of detecting the interaction of a first and a second 20 molecule, said method comprising:
 - i) providing a first molecule having a first fluorophore attached thereto;
 - ii) providing a second molecule having a second fluorophore attached thereto wherein said first fluorophore and said second fluorophore are the same species of fluorophore and, when juxtaposed, form an H-dimer thereby quenching fluorescence produced by the fluorophores; and
 - iii) detecting a change in fluorescence or absorbance produced by said fluorophores where a decrease in fluorescence or a change in absorbance indicates that the first molecule and the second molecule are interacting.

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- 5 86. The method of claim 84, wherein said fluorophore is linked to said first molecule by a linker.
 - 87. The method of claim 84, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
- The method of claim 84, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
 - 89. A method of detecting a change in conformation or cleavage of a macromolecule, said method comprising:
 - i) providing a macromolecule having attached thereto two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and
 - ii) detecting a change in fluorescence or absorbance of said fluorophores wherein a change in fluorescence or absorbance indicates a change in conformation or cleavage of said macromolecule.
- 90. The method of claim 85, wherein said macromolecule is selected from the group consisting of a polypeptide, a nucleic acid, a lipid, a polysaccharide, and an oligosaccharide.
 - 91. The method of claim 85, wherein said macromolecule is attached to a solid support.
- 25 92. The method of claim 85, wherein said macromolecule is inside a mammalian cell.
 - 93. The method of claim 85, wherein said macromolecule bears a hydrophobic group.



- bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).
 - 95. The method of claim 85, , wherein said fluorophores are linked to the macromolecule by linkers.
 - 96. The method of claim 85, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
 - 97. The method of claim 85, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
 - 98. The method of claim 85, wherein said contacting is in a histological section.
 - 99. The method of claim 85, wherein said contacting is in a cell culture.
 - 100. The method of claim 85,, wherein said contacting is contacting a seeded or cultured adherent cell.
- 25 101. The method of claim 85,, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.





102. The method of claim 85, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, fluorescence microplate reader, flow cytometry, fluorometry, confocal microscopy, and absorption spectroscopy.